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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
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OFFICE OF PREVENTION,
PESTICIDES, AND TOXIC SUBSTANCES

TXR No.: 0054809

MEMORANDUM

DATE: February 29, 2008

SUBJECT: **THIENCARBAZONE-METHYL:** Report of the Cancer Assessment Review Committee

PC Code: 015804

FROM: Jessica Kidwell, Executive Secretary Jessica Kidwell
Cancer Assessment Review Committee
Health Effects Division (7509P)

TO: John Doherty, Toxicologist (RRB3)
Will Donovan, Risk Assessor (RRB3)
Health Effects Division (7509P)

Hope Johnson, PM
Herbicide Branch, Registration Division (7505P)

The Cancer Assessment Review Committee met on January 9, 2008 to evaluate the carcinogenic potential of Thiencarbazone-methyl. Attached please find the Final Cancer Assessment Document.

Rcvd in RIC 3/18/08 CA

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

THIENCARBAZONE-METHYL

PC Code: 015804

FINAL
February 29, 2008

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

Thiencarbazone-methyl (TCM) Cancer Assessment Document Final

DATA PRESENTATION: John Doherty, Toxicologist

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise noted.)

Gregory Akerman

Karlyn Bailey

Lori Brunsman, Statistician

William Burnam, Chair

Marion Copley

Vicki Dellarco

Ray Kent

Mary Manibusan — Jessica Kidwell for MM.

Nancy McCarroll

Rob Mitkus

Esther Rinde

Jess Rowland

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist — See Attached Sheet

OTHER ATTENDEES: Jim Tompkins (RD/HB), Edward Scollon (HED/RAB2), Nader Tadayon (HED/RRB3), Cathy Eiden (HED/RRB3), Conference call with PMRA Canada (Charles Smith, Tanya Clegg, Carmen Chung, Catherine Newfield, Cathering Adcock) and United Kingdom (David Andrew)

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John Doherty, Toxicologist

DOCUMENT PREPARATION: _____
Jessica Kidwell, Executive Secretary

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EXECUTIVE SUMMARY

On January 9, 2008, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met to evaluate the carcinogenic potential of two chemicals, thiencarbazone-methyl (TCM), an herbicide and cyprosulfamide (CS), a safener. Registration for both chemicals is sponsored by the Bayer Company. TCM and CS, which will be applied together, are being jointly reviewed by the United Kingdom, Canada and the USA. The United Kingdom has the lead for the review of the toxicity data base and HED has the responsibility for the secondary review of the toxicity data base. This report presents the background deliberations and results for TCM only. The background and outcome of the carcinogenicity evaluation of CS is presented in a separate document (TXR No. 0054810).

John Doherty of Reregistration Action Branch 3 presented the chronic toxicity/carcinogenicity studies in Wistar rats and the carcinogenicity study in C57BL/6J mice. TCM was administered in the diet to 60 male and 60 female Wistar rats per dose group, in doses of 0, 500, 2500 or 5000 ppm (main groups) for a scheduled treatment period of 2 years (carcinogenicity part of the study). TCM was also administered in the diet to 10 male and 10 female rats per dose group, in doses of 0, 200, 500, 2500 or 5000 ppm (satellite groups) for a treatment period of 1 year (chronic part of the study). Treatment resulted in the following mean daily test substance intake for main groups (averaged over the study period of two years and given in ascending dosages): 22.8, 115.2 and 234.0 mg/kg body weight for males and 29.9, 152.9 and 313.4 mg/kg for females. During their treatment period of one year mean daily test substance intake of satellite group animals was for the males: 10.6, 27.2, 136.4 and 268.60 mg/kg body weight and for the females: 13.2, 35.8, 176.7 and 366.6 mg/kg. Groups of 60 male and 60 female C57BL/6J mice were fed diet containing 0, 200, 1000 or 4000 ppm of TCM for at least 28 weeks. After 28 weeks, 10 males and 10 females from each group allocated to the 28-week phase of the study were necropsied at the scheduled interim sacrifice. The remaining 50 animals/sex/group were allocated to the final sacrifice group of the carcinogenicity phase of the study. Final sacrifice occurred after at least 78 weeks of treatment. The mean intake of TCM over 78 weeks was calculated to be 0, 29.2, 147 and 599 mg/kg/day in males and 0, 36.8, 185 and 758 mg/kg/day in females, at 0, 200, 1000 and 4000 ppm, respectively. He also presented information on mutagenicity, structure activity relationships, and mode of action.

The CARC concluded the following:

Carcinogenicity

Rat

• There were no significant treatment-related increases in tumors in male or female Wistar rats.

• Adequacy of Dosing: No overt toxicity was seen up to the highest dose tested of 5000 ppm (234 and 313.4 mg/kg/day in males and females, respectively), aside from a possible decrease in

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serum triglycerides, raising a question that the doses were inadequate for carcinogenicity assessment. However, based on the results of the subchronic study with TCM, the doses selected for the definitive carcinogenicity assessment are considered appropriate since there was significant toxicity at 7000 ppm in the rat subchronic study. This toxicity included treatment related mortality, clinical chemistry (increased alkaline phosphatase activity in males) and urinalysis (crystals in urine), as well as non-neoplastic changes including intrapelvic eosinophilic urolithiasis of the kidney and bladder and urothelial hyperplasia of the bladder. It is noted that an effect on serum triglycerides was not seen at the higher dose of 7000 ppm although the duration of exposure was for only 90 days. The CARC, therefore, concluded that the top dose of 5000 ppm in the cancer study was approaching an adequate dose based on the significant toxicity seen at 7000 ppm in the subchronic rat study. Furthermore, the CARC believed that testing at higher doses would not change the conclusions for assessing carcinogenicity.

Mouse

Males

In males, the censored incidences of transitional cell tumors of the urinary bladder and urethra/prostate for the 0, 200, 1000, and 4000 ppm dose groups, respectively were as follows:

Bladder papillomas:	0/39 (0%), 0/34 (0%), 0/39 (0%), 1/28 (4%)
Urethra carcinomas:	0/38 (0%), 0/34 (0%), 0/37 (0%), 1/28 (4%)
Combined:	0/38 (0%), 0/34 (0%), 0/37 (0%), 2/28 (7%)

There were significant increasing trends for papillomas and carcinomas (both at $p<0.05$) and combined ($p<0.01$), as well as a significant difference in the pair wise comparison of the 4000 ppm dose group with the controls for the combined tumors, at $p<0.05$. These are considered to be rare tumors. Significant non-neoplastic pathology (hyperplasia and inflammation) occurred in males at the high dose. Therefore, the CARC considered the transitional cell tumors at the high dose to be treatment-related.

Females

In females, the censored incidences of transitional cell tumors of the urinary bladder for the 0, 200, 1000, and 4000 ppm dose groups, respectively were as follows:

Papillomas:	0/45 (0%), 0/45 (0%), 0/46 (0%), 2/48 (4%)
Carcinomas:	0/45 (0%), 0/45 (0%), 0/46 (0%), 1/48 (2%)
Combined:	0/45 (0%), 0/45 (0%), 0/46 (0%), 3/48 (6%)

There was a significant increasing trend for combined tumors at $p<0.05$. These are considered to be rare tumors. Significant non-neoplastic pathology (hyperplasia and inflammation) occurred in females at the high dose. Therefore, the CARC considered the transitional cell tumors at the high

dose to be treatment-related.

Adequacy of Dosing: The CARC concluded that while the high dose may be an excessive dose, the study is considered adequate because the effects seen in both sexes at the high dose are typical of the primary target site (urothelial toxicity in the kidney and bladder) for this class of chemicals.

Mutagenicity

•There is no mutagenicity concern for TCM or its major metabolites.

Structure Activity Relationship.

• TCM does not have a structural alert for genotoxicity. However, TCM is related to sulfonamides and sulfoamido compounds which have been shown to induce urinary bladder tumors at high doses similar to the response seen with TCM in the mouse study.

Mode of Action

• The mode of action for the induction of the transitional cell tumors in the bladder in males (one incident), urethra/prostate tumor (one incident) and transitional cell papilloma (2 incidents) and carcinoma (one incident) in the bladder of females is considered to be related to the secondary effects of urothelial toxicity (irritation) and regenerative proliferation associated with the formation of urinary tract crystals/calculi in the urothelial structures at higher doses. This is a well established MOA for bladder tumors. TCM is not considered to be mutagenic.

Classification and Quantification of Carcinogenic Potential

In accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified TCM as **"Not Likely to be Carcinogenic to Humans" at doses that do not cause urothelium cytotoxicity.** The formation of the low incidence of the transitional cell tumors of the bladder in both sexes and urethra/prostate in males seen at the high dose in mice is considered to be related to the secondary effects of the urothelial toxicity (irritation) and regenerative proliferation associated with the formation of urinary tract crystals/calculi. This is a common mode of action for bladder carcinogenesis in rodents for non-genotoxic chemicals. No tumors were seen in rats. There is also no concern for mutagenicity.

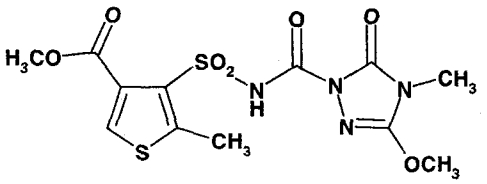
Quantification is not required. The chronic Reference Dose (cRfD) would be protective of both cancer and non cancer effects.

I. INTRODUCTION

On January 9, 2008, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs (OPP) met to evaluate the carcinogenic potential of two chemicals, thiencarbazone-methyl (TCM), an herbicide and cyprosulfamide (CS), a safener. Registration for both chemicals is sponsored by the Bayer Company. This report presents the background deliberations and results for TCM only. The background and outcome of the carcinogenicity evaluation of CS is presented in a separate document (TXR No. 0054810).

II. BACKGROUND INFORMATION

Thiencarbazone methyl (TCM, BYH 18636, methyl 4-[[[(4,5-dihydro-3-methoxy-4-methyl-5-oxo-1H-1,24-triazol-1-yl)carbonyl]amino]sulfonyl]-5-methyl-3-thiophenecarboxylate) is a new active ingredient herbicide proposed for use on agricultural commodities corn (field, white, sweet and popcorn) and wheat and for terrestrial non-food crop ornamentals (shrubs, trees, flowers and foliage plants) in outdoor landscapes and turf. TCM will be applied with the safener cycloprosulfamide (CS).



III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Reference: BYH 18636 Combined Chronic/Oncogenicity Study in Wistar Rats *Dietary Administration for 2 Years).Bayer HealthCare AG, Wuppertal, Germany, Laboratory Report No.: ATO3629. Dated 01/30/07. MRID No. 47070134.

A. Experimental Design:

TCM (BYH 18636,m ix-batch 702-73-06-0001, 96.0 - 96.4% of purity) was administered in the diet to 60 male and 60 female Wistar rats per dose group, in doses of 0, 500, 2500 or 5000 ppm (main groups) for a scheduled treatment period of 2 years (carcinogenicity part of the study). Furthermore, TCM was administered in the diet to 10 male and 10 female rats per dose group, in doses of 0, 200, 500, 2500 or 5000 ppm (satellite groups) for a treatment period of 1 year

(chronic part of the study). Treatment resulted in the following mean daily test substance intake for main groups (averaged over the study period of two years and given in ascending dosages): 22.8, 115.2 and 234.0 mg/kg body weight for males and 29.9, 152.9 and 313.4 mg/kg for females. During their treatment period of one year mean daily test substance intake of satellite group animals was for the males: 10.6, 27.2, 136.4 and 268.60 mg/kg body weight and for the females: 13.2, 35.8, 176.7 and 366.6 mg/kg.

B. Discussion of Tumor Data

There were no significant treatment-related increases in tumors in male or female rats.

C. Non-Neoplastic Lesions in the Kidney and Urinary Bladder

There were no significant non-neoplastic lesions in this chronic feeding carcinogenicity study with rats in the kidney, bladder or other structures of the urinary tract in the definitive chronic feeding/carcinogenicity study in rats.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

There were no effects of treatment at even the highest test dose of 5000 ppm (234 and 313.4 mg/kg/day in males and females, respectively), aside from a possible decrease in serum triglycerides, raising a question that the doses were inadequate for carcinogenicity assessment. However, based on the results of the subchronic study with TCM, the doses selected for the definitive carcinogenicity assessment are considered appropriate since there was significant toxicity at 7000 ppm in the rat subchronic study (MRID No. 47040126). This toxicity included treatment related mortality, clinical chemistry (increased alkaline phosphatase activity in males) and urinalysis (crystals in urine), as well as non-neoplastic changes including intrapelvic eosinophilic urolithiasis of the kidney and bladder and urothelial hyperplasia of the bladder. It is noted that an effect on serum triglycerides was not seen at the higher dose of 7000 ppm although the duration of exposure was for only 90 days. The CARC, therefore, concluded that the top dose of 5000 ppm in the cancer study was approaching an adequate dose based on the significant toxicity seen at 7000 ppm in the subchronic rat study. Furthermore, the CARC believed that testing at higher doses would not change the conclusions for assessing carcinogenicity.

2. Carcinogenicity Study in Mice

Reference: BYH 18636, Carcinogenicity study of BYH 18636 in the C57BL/6J mouse by dietary administration. Bayer Crop Science SA, Sophia Antipolis, France, Report No.: SA 04062, 11/10/2006. MRID No.: 47070135.

A. Experimental Design

Groups of 60 male and 60 female C57BL/6J mice were fed diet containing 0, 200, 1000 or 4000 ppm of TCM (BYH 18636, mix-batch 702-73-06-0001) for at least 28 weeks. After 28 weeks, 10 males and 10 females from each group allocated to the 28-week phase of the study were necropsied at the scheduled interim sacrifice. The remaining 50 animals/sex/group were allocated to the final sacrifice group of the carcinogenicity phase of the study. Final sacrifice occurred after at least 78 weeks of treatment. The mean intake of TCM over 78 weeks was calculated to be 0, 29.2, 147 and 599 mg/kg/day in males and 0, 36.8, 185 and 758 mg/kg/day in females, at 0, 200, 1000 and 4000 ppm, respectively. Mortality and clinical signs were checked daily. Additionally, detailed physical examinations including palpation for masses were performed weekly throughout treatment. Body weight and food consumption were measured weekly for the first 13 weeks of the study, then monthly thereafter. Haematology determinations were performed at approximately 6, 12 and 18 months from designated animals. Where possible, blood smears were prepared from moribund animals just before sacrifice. All animals were subjected to necropsy, with selected organs weighed at scheduled interim and final sacrifice. Designated tissues were fixed and examined microscopically. In addition, urinary bladder stones sampled from five males at 4000 ppm were analyzed for the presence of test substance.

B. Discussion of Survival and Tumor Data

Survival Analysis

Male mice showed a statistically significant increasing trend for mortality with increasing doses of TCM, as well as a significant pair-wise comparison of the 4000 ppm dose group with the controls, both at $p < 0.05$. There was not a statistically significant trend in mortality with increasing doses of TCM in female mice, however, there was a statistically significant pair-wise comparison of the 200 ppm dose group with the controls at $p < 0.05$ (Tables 1 and 2).

Table 1. Thiencarbazone-Methyl – C57BL/6J Mouse Study (MRID 47070135)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-28	29[i]	29-53	54-80[f]	Total
0	0/60	10/60	4/50	7/46	11/50 (22)*
200	4/60	9/56	1/47	12/46	17/51 (33)
1000	1/60	9/59	3/50	8/47	12/51 (24)
4000	1/60	10/59	6/49	15/43	22/50 (44)*

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 29.
[f]Final sacrifice at weeks 78-80.

( ) Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Thiencarbazone-Methyl – C57BL/6J Mouse Study (MRID 47070135)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Weeks

Dose (ppm)	1-28	29[i]	29-53	54-80[f]	Total
0	0/60	10/60	3/50	3/47	6/50 (12)
200	2/60	10/58	2/48	10/46	14/50 (28)*
1000	1/60	10/59	1/49	2/48	4/50 (8)
4000	0/60	10/60	1/50	8/49	9/50 (18)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 29.
[f]Final sacrifice at weeks 78-80.

( ) Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analyses

Male mice had statistically significant trends in bladder transitional cell papillomas and carcinomas, both at $p < 0.05$. There was also a statistically significant trend at $p < 0.01$, and a significant pair-wise comparison of the 4000 ppm dose group with the controls at $p < 0.05$, for bladder transitional cell papillomas and carcinomas combined. Female mice had a statistically significant trend in bladder transitional cell papillomas and carcinomas combined at $p < 0.05$, however, female mice had no significant pair-wise comparisons of the dosed groups with the controls. The statistical analyses of the tumors in the male mice were based upon Peto's Prevalence Test. The statistical analyses of the tumors in the female mice were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Tables 3 and 4).

Table 3. Male Urinary Bladder and Urethra/Prostate Transitional Cell Tumor Rates[+] and Peto's Prevalence Test Results (From L. Brunsman memo December 12, 2007, TXR No.: 0054792)

	Dose (ppm)			
	0	200	1000	4000
Bladder Papillomas (%)	0/39 (0)	0/34 (0)	0/39 (0)	1[a]/28 (4)
p =	0.02686*	-	-	0.11896
Urethra Carcinomas (%)	0/38 (0)	0/34 (0)	0/37 (0)	1[b]/28 (4)
p =	0.02835*	-	-	0.12202
Combined (%)	0/38 (0)	0/34 (0)	0/37 (0)	2/28 (7)
p =	0.00342**	-	-	0.04842*

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First urinary bladder transitional cell papilloma observed at week 80, dose 4000 ppm, in a final sacrifice animal.
[b]First urethra/prostate transitional cell carcinoma observed at week 79, dose 4000 ppm, in a final sacrifice animal.

Table 4. Female Bladder Transitional Cell Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results (From L. Brunsman memo December 12, 2007, TXR No.: 0054792).

	Dose (ppm)			
	0	200	1000	4000
Papillomas (%)	0/45 (0)	0/45 (0)	0/46 (0)	2[a]/48 (4)
p =	0.06700	1.00000	1.00000	0.26367
Carcinomas (%)	0/45 (0)	0/45 (0)	0/46 (0)	1[b]/48 (2)
p =	0.2609	1.00000	1.00000	0.51613
Combined (%)	0/45 (0)	0/45 (0)	0/46 (0)	3/48 (6)
p =	0.01693*	1.00000	1.00000	0.13329

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First papilloma observed at week 79, dose 4000 ppm, in a final sacrifice animal.
[b]First carcinoma observed at week 79, dose 4000 ppm, in a final sacrifice animal.

Note: (For both tables):
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions

Tables 5-9 illustrate the presence of non-neoplastic pathology. At the microscopic examination, treatment-related effects were found in the urinary bladder, kidney, prostatic urethra, ureter, skin and bone marrow. [Note: These tables and the associated commentary were taken from the study summary prepared by the testing laboratory and the Bayer Company but were verified by the United Kingdom reviewer.]

In the urinary bladder, histological confirmation of the stones observed macroscopically was made with the males being more affected than females. The presence of the following various stone-induced findings, secondary to chronic irritation, was observed in both sexes:

hyperplastic changes (simple and/or nodular/glandular urothelial hyperplasia),

- inflammatory changes (interstitial oedema, suburothelial and/or serosal mixed cell infiltrate, intramuscular inflammatory cell infiltrate and induced arteritis), focal/multifocal adenomyosis in a few treated males.

Table 5: Incidence and severity of microscopic changes in the urinary bladder, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	49	49	50	50	48	49	47	49
Stone(s): intraluminal								
Minimal	0	0	0	6	0	0	0	2
Slight	0	0	0	1	0	0	0	3
Moderate	0	0	0	13	0	0	0	2
Marked	0	0	0	11	0	0	0	5
Severe	0	0	0	1	0	0	0	3
Total	**0**	**0**	**0**	**32****	**0**	**0**	**0**	**15****
Stone(s): only noted at necropsy	**1**	**0**	**0**	**9***	**0**	**0**	**0**	**5***
Total incidence of animals with stones	**1**	**0**	**0**	**41****	**0**	**0**	**0**	**20****
Urothelial hyperplasia: simple: multifocal/diffuse								
Minimal	0	0	1	14	0	0	0	10
Slight	0	0	0	22	0	0	0	10
Moderate	0	0	0	2	0	0	0	0
Total	**0**	**0**	**1**	**38****	**0**	**0**	**0**	**20****
Urothelial hyperplasia: nodular/glandular: multifocal/diffuse								
Minimal	0	0	0	12	0	0	0	2
Slight	0	0	0	9	0	0	0	4
Moderate	0	0	0	2	0	0	0	6
Marked	0	0	0	0	0	0	0	1
Total	**0**	**0**	**0**	**23****	**0**	**0**	**0**	**13****
Interstitial oedema: diffuse								
Minimal	0	0	0	16	0	0	0	8
Slight	0	0	0	12	0	0	0	5
Moderate	0	0	0	6	0	0	0	0
Total	**0**	**0**	**0**	**34****	**0**	**0**	**0**	**13****
Suburothelial mixed cell infiltrate: focal/multifocal								
Minimal	1	0	0	21	1	0	0	7
Slight	0	0	0	18	0	0	0	9
Moderate	0	0	0	2	0	0	0	3
Total	**1**	**0**	**0**	**41****	**1**	**0**	**0**	**19****
Intramuscular inflammatory cell infiltrate: focal/multifocal								
Minimal	0	0	0	24	0	0	0	12
Slight	0	0	0	10	0	0	0	7
Total	**0**	**0**	**0**	**34****	**0**	**0**	**0**	**19****

Serosal mixed cell infiltrate: focal/multifocal								
Minimal	0	0	0	3	0	0	0	5
Slight	0	0	0	3	0	0	0	1
Total	**0**	**0**	**0**	**6***	**0**	**0**	**0**	**6***
Induced arteritis								
Minimal	0	0	0	4	0	0	0	3
Total	**0**	**0**	**0**	**4**	**0**	**0**	**0**	**3**
Adenomyosis: focal/multifocal								
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Total	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**

*: $p \leq 0.05$; **: $p \leq 0.01$

In the kidney, a higher incidence and severity of unilateral and/or bilateral pelvic dilatation in both sexes was observed at 4000 ppm. This finding was considered to be secondary to the stone-induced urinary obstruction.

Table 6: Incidence and severity of microscopic changes in the kidney, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	50	50	50	50	50	50	49	50
Pelvic dilatation: unilateral								
Minimal	0	0	0	5	0	0	1	3
Slight	1	0	0	3	1	1	0	1
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	1	0
Severe	0	0	1	0	0	0	0	1
Total	**1**	**0**	**1**	**8***	**1**	**1**	**2**	**6**
Pelvic dilatation: bilateral								
Minimal	0	1	0	3	0	0	0	2
Slight	0	0	0	4	0	0	0	1
Total	**0**	**1**	**0**	**7***	**0**	**0**	**0**	**3**
Pelvic dilatation: unilateral/bilateral								
Minimal	0	1	0	8	0	0	1	5
Slight	1	0	0	7	1	1	0	2
Moderate	0	0	0	0	0	0	0	1
Marked	0	0	0	0	0	0	1	0
Severe	0	0	1	0	0	0	0	1
Total	**1**	**1**	**1**	**15****	**1**	**1**	**2**	**9****

*: $p \leq 0.05$; **: $p \leq 0.01$

In the prostatic urethra, minimal to moderate urothelial hyperplasia was observed at 4000 ppm in males and was considered to be treatment-related.

Table 7: Incidence and severity of microscopic changes in the urethra (prostate), all males, carcinogenicity phase

Sex	Males			
Dose level (ppm)	0	200	1000	4000
Number of animals	49	50	48	50
Urothelial hyperplasia: urethra				
Minimal	0	0	0	3
Slight	0	0	0	1
Moderate	0	0	0	1
Total	**0**	**0**	**0**	**5***

*: $p \leq 0.05$

In the ureter (preserved when macroscopic findings were present at necropsy), simple urothelial hyperplasia was observed in 2/9 males at 4000 ppm and was considered to be treatment-related.

In the skin, a statistically significantly higher incidence of chronic ulcerative dermatitis was observed in males at 4000 ppm. Chronic ulcerative dermatitis is a spontaneous disease commonly observed in the C57BL/6J mouse. As this finding was located in the anogenital region or surrounding area, it was considered to be probably related to a stone-induced dysuria and thus indirectly treatment-related.

Table 8: Incidence and severity of microscopic changes in the skin, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	49	48	49	48	48	50	48	47
Chronic ulcerative dermatitis								
Slight	0	0	1	0	1	1	0	0
Moderate	0	2	1	7	0	2	0	0
Marked	1	3	1	3	0	0	0	0
Total	**1**	**5**	**3**	**10***	**1**	**3**	**0**	**0**

*: $p \leq 0.05$

In the bone marrow, a higher incidence and severity (males only) of myeloid hyperplasia was observed in both sexes at 4000 ppm. This finding was considered to be secondary to the chronic inflammation of the urinary bladder and chronic ulcerative dermatitis.

Table 9: Incidence and severity of microscopic changes in the bone marrow, sternum, all animals, carcinogenicity phase

Sex	Males				Females			
Dose level (ppm)	0	200	1000	4000	0	200	1000	4000
Number of animals	50	50	50	50	50	50	49	50
Myeloid hyperplasia: diffuse								
Minimal	2	1	1	9	3	3	4	7
Slight	1	2	0	2	2	0	0	2
Moderate	1	2	0	6	0	0	0	0
Marked	0	1	0	0	0	0	0	0
Total	**4**	**6**	**1**	**17***	**5**	**3**	**4**	**9**

*: $p \leq 0.05$

All other non neoplastic findings were those commonly observed in this strain and age of mouse kept under monitored environmental conditions and were considered to be incidental in origin.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The CARC concluded that while the high dose may be an excessive dose, the study is considered adequate because the effects seen in both sexes at the high dose are typical of the primary target site (urothelial toxicity in the kidney and bladder).

IV. TOXICOLOGY

1. Metabolism

In a series of metabolism studies (MRIDS 47070201 and 47070150, both 2006) from 91-99% of administered radiolabelled (either thiophene or dihydrotriazole ^{14}C positions) TCM was recovered. About 50% was in the urine and 50% in the feces meaning that about 50% was absorbed from the gastrointestinal tract since only a small amount (~1.4%) was shown to be excreted via the bile. Excretion via CO_2 was negligible (0.01%). Little remained in the carcass (0.5 to 0.7%). There was no indication that the kidney or bladder retained radioactivity in these short-term exposure studies. In studies with the dihydrotriazole labeled parent, the thyroid was noted to retain a relatively high concentration (0.0271 µg/gm).

TCM was the not extensively metabolized and parent compound was 81-92% of the dose recovered. Studies with thiophene labeled parent indicated a sulfonamide-carboxylic acid was recovered as a minor metabolite (1-2% of the dose) and the chemical thienosaccharine was also detected (0.1 - 0.2% of the dose, see structure in section IV.3 below). Metabolism proceeds by cleavage of the urea group and hydrolysis of the methyl ester that is followed by re-cyclization to an intramolecular sulfonamide (thienosaccharine). Studies with labeled dihydrotriazole also indicated low metabolism (91% recovered as parent with 5 trace metabolites less than 1% each) and metabolism proceeds via hydrolysis to yield the dihydrotriazole moiety that is subsequently demethylated and converted to methyl-carbamate.

2. Mutagenicity

TCM or its principal metabolites (sulfonamide, desmethyl and carboxylic acid) were not found to be mutagenic or to demonstrate genetic toxicity in the battery of studies as shown in the Table 10.

Table 10. Thiencarbazone-methyl and Metabolites- Mutagenicity Studies

Study	MRID	Results	Classification: UK/HED[a]
Thiencarbazone-methyl			
870.5100.Bacterial – Ames test/AT03630	47070136 (2007)	Negative up to 512 ug/plate in TA 1535, TA 100, TA 1537, TA 98 and TA 102. Antibacterial effect > 30 ug/plate.	[Reliable with restrictions]/ Acceptable/Guideline
870.5100.Bacterial – Ames test/AT02274	47070137 (2005)	Negative up to 400 ug/plate. Antibacterial at > 30 ug/plate.	[Reliable with restrictions]/ Acceptable/Guideline
870.5300.Forward mutation *In vitro*/AT003686	47070140 (2007)	Negative in the V79/HPRT forward mutation test at 600 ug/mL	[Totally reliable]/ Acceptable/Guideline
870.5300.Forward mutation *In vitro*/ ATO2752	47070141 (2005)	Negative (same as above).	[Reliable with restrictions]/ Acceptable/Guideline
870.5375. *In vitro* chromosome aberration test in CHO/AT)3625	47070144 (2007)	No evidence of clastogenic effect at doses up 400 ug/ml.	[Totally reliable]/ Acceptable/Guideline
870.5375. *In vitro* chromosome aberration test in CHO/AT)2499	47070145 (2005)	Same as above.	[Totally reliable]/ Acceptable/Guideline
870.5395. Mouse *in vivo* micronucleus test/AT01568	47070214 (2004)	No indications of clastogenic effect at doses up to 500 mg/kg (two doses, ip)	[Totally reliable]/ Acceptable/Guideline
Sulfanomide metabolite			
80.5100.Bacterial – Ames//AT03605	47040208 (2006) BYH- 18636 – sulfonamide	Not antibacterial, Not mutagenic at up to 7000 ug/plate.	[Totally reliable]/ Acceptable/Guideline
Desmethyl metabolite			
870.5100.Bacterial – Ames/ATO3497	47070138 (2006) BYH – 18636 – desmethyl	Antibacterial at 500 ug/plate, Not mutagenic at up to 5000 ug/plate.	[Totally reliable]/ Acceptable/Guideline
870.5300.Forward mutation *In vitro* /ATOAT03687	47070142 (2007) BYH – 18636 – desmethyl	Negative	[Totally reliable]/ Acceptable/Guideline
870.5375. *In vitro* chromosome aberration	47070146 (2007) BYH – 18636 –	No clastogenic effect at up to 1300 ug/ml.	[Totally reliable]/ Acceptable/Guideline

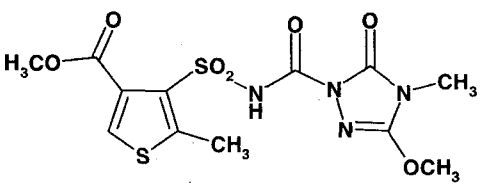
Table 10. Thiencarbazone-methyl and Metabolites- Mutagenicity Studies			
Study	**MRID**	**Results**	**Classification: UK/HED[a]**
test in CHO/AT03678	desmethyl		
Carboxylic acid metabolite			
870.5100.Bacterial – Ames/ ATO1522A	47070139 (2004/2006) BYH – 18636 – carboxylic acid	Not mutagenic at up to 5000 ug/plate, Antibacterial at 1581 ug/plate.	[Totally reliable]/ Acceptable/Guideline
870.5300.Forward mutation *In vitro*/ AT02038	47070143 BYH – 18636 – carboxylic acid	Negative	[Totally reliable]/ Acceptable/Guideline
870.5375. *In vitro* chromosome aberration test in CHO/AT01980	47070147 (2005) BYH – 18636 – carboxylic acid	No clastogenic effect at up to 1200 ug/ml.	[Totally reliable]/ Acceptable/Guideline

[a] The HED classification is based on the secondary reviews as provided by N. McCarroll (personal communication sent via e-mail December 13, 2007).

Fourteen genetic toxicology studies on thiencarbazone-methyl (TCM, 7 studies) and its major metabolites (sulfonamide metabolite, 1 study; desmethyl metabolite, 3 studies; and carboxylic acid metabolite, 3 studies) have been submitted and found to be acceptable for regulatory purposes. Results indicate that the parent compound, TCM was not mutagenic in *Salmonella typhimurium* TA1535, TA100, TA1537 or TA98 or in the cultured V79 Chinese hamster lung fibroblast cell line. It was also not clastogenic *in vitro* in cultured V79 Chinese hamster lung fibroblast cells or clastogenic or aneugenic *in vivo* in the mouse micronucleus assay. Similarly, the sulfonamide, desmethyl, or carboxylic acid metabolites did not induce reverse gene mutations in the *S. typhimurium* tester strains previously mentioned. Additional testing with the latter two metabolites showed no induction of forward gene mutations or chromosome aberrations in V79 cells. All of the above *in vitro* assays were conducted in the absence and the presence of an exogenous metabolic activation (S9) system. Based on these considerations, it is concluded that there is no mutagenic concern for TCM or its major metabolites.

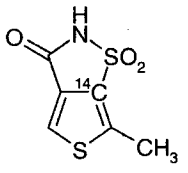
3. Structure-Activity Relationships

The structure of thiencarbazone methyl is depicted below.



TCM does not have a structural alert for genotoxicity. However, TCM is related to sulfonamides

and sulfoamido compounds which have been shown to induce urinary bladder tumors at high doses similar to the response seen with TCM in the mouse study. TCM metabolism ultimately results in the formation of thienosaccharine which differs from saccharine by having a methyl group on the thiophene ring. Saccharine has been implicated in formation of bladder tumors. The formation of thienosaccharine from thiencarbazone methyl in rats is relatively minor being only 0.1 to 0.2% % of the administered radioactive parent (see metabolism section above).



thienosaccharine.

4. Subchronic and Chronic Toxicity

1) Subchronic Toxicity

a) Rat Study (MRID No: 47070126)

Executive summary: (As prepared by the testing laboratory and the Bayer Corporation). BYH 18636 (batch number NLL6954-10, 98% w/w purity) was administered continuously via the diet to separate groups of Wistar rats (10/sex/group) at dose levels of 0, 400, 2 000 and 7 000 ppm equivalent to 24.7, 123 and 439 mg/kg/day in males and 30.8, 154 and 543 mg/kg/day in females, respectively, for at least 90 days. An additional, 10 males and 10 females fed either 0 or 7 000 ppm of test diet for at least 90 days were maintained on control diet for a further 30 days to examine the reversibility of any effects seen.

At 7000 ppm, one treatment-related mortality was recorded on Day 49 of the dosing phase. Clinical signs observed for this animal prior to death were a red soiled anogenital region and red coloured urine and macroscopic examination revealed that the probable cause of death was a urinary tract obstruction. At clinical chemistry evaluation, a tendency towards higher alkaline phosphatase activity was seen in male animals (+25%) at the end of the dosing phase only. At urinalysis, sulfonamide-like crystals were seen in the urine of 9/10 males and 10/10 females. The presence of these crystals in large amounts correlated with a cloudy appearance observed in the urine. Neither of these effects was observed at the end of the recovery phase of the study. At microscopic examination at the end of the dosing phase, treatment-related effects were observed in the kidney and urinary bladder in both male and female animals. Intrapelvic eosinophilic urolithiasis within the kidneys was found in 3/10 males and in 1/10 females. A similar eosinophilic urolithiasis was observed within the lumen of the urinary bladder in 2/10 males, this was correlated with gritty content (stones) observed macroscopically in 3/10 males. Within the urinary bladder, urothelial hyperplasia was found in 3/10 males and 1/10 females. Slight to mild collecting duct hyperplasia was found in 4/10 males and 2/10 females. Following the recovery period, treatment-related changes were again identified within the kidney and the urinary bladder

in males and females at 7 000 ppm but at a lower frequency than at the end of the treatment phase. In the kidney, abnormal intrapelvic eosinophilic urolithiasis was found in 1/10 females. Hyperplasia in the collecting ducts was found in 1/8 males and 1/10 females, the affected male also had a mild simple diffuse urothelial hyperplasia in the urinary bladder.

At 2,000 ppm, there were no adverse treatment related findings observed in either sex but at the end of the treatment phase of the study, sulfonamide-like crystals were seen in the urine of 3/10 males and 4/10 females.

At 400 ppm, no treatment related findings were observed in either sex.

The No Observed Adverse Effect Level (NOAEL) in the Wistar rat when administered BYH 18636 in the diet over a 90-day period was 2 000 ppm (equivalent to 123 mg/kg/day for males and 154 mg/kg/day for females).

Classification:

By the United Kingdom reviewer: Totally reliable. The reviewer commented on possible incomplete preparation of the brain with regard to representative regions not being examined histologically since the study reports only that the brain was assessed. Other comments made by the reviewer included an increase in plasma alkaline phosphatase, lower pituitary weight (no dose response) and lower testis weight. The study uses a test material of higher purity than the currently manufactured material.

By the HED reviewer: ACCEPTABLE/GUIDELINE. The study satisfies the requirement for a series 870.3100 oral subchronic study in rats. HED concurs with the assignment of 2000 ppm as the NOAEL and 7000 ppm for the LOAEL. The possible effects on testis weight and plasma alkaline phosphatase are noted but not critical in assigned of the NOAEL. The assessment of histopathology of the brain with regard to representative regions not being examined is noted but is not considered a major study deficiency for the study. There is also a subchronic neurotoxicity study (2006, MRID No.: 47040149) determined by the United Kingdom reviewer to be "totally reliable".

b) Mouse Study*: (2004, MRID No.: 47070129)*

Executive summary: (As prepared by the testing laboratory and the Bayer Corporation). BYH 18636 (batch number NLL6954-10, 98.0 to 99.7 % w/w purity), was administered continuously via the diet to groups of C57BL/6 mice (10/sex/group) at concentrations of 0, 500, 2 000 and 4 000 ppm for at least 90 days (equating approximately to 0, 76, 315 and 637 mg/kg/day in males and 0, 103, 409 and 789 mg/kg/day in females).

At 4 000 ppm, the only treatment-related finding was a urinary bladder calculus observed at autopsy in one male. This finding was accompanied by marked submucosal inflammatory cell infiltration,

minimal diffuse urothelial inflammation and moderate diffuse urothelial hyperplasia of urinary bladder.
At 2 000 and 500 ppm, no treatment-related findings were observed in either sex.

The NOEL of BYH 18636 in this study was 2 000 ppm in males (equating to 315 mg/kg/day) and 4 000 ppm in the females (equating to 789 mg/kg/day).

Classification: UK Reviewer: "Reliable with restrictions" Restrictions involve histopathology of the brain and OECD guidelines preference for cerebrum, cerebellum and medulla pons. Study also uses a test material of higher purity than current manufactured product.

HED reviewer: Acceptable/Guideline. The study satisfies the 870.3100 guideline requirement for a carcinogenicity study in the mouse.

2) Chronic Toxicity

a) Rat Study (MRID 47070134)

Executive summary:

BYH 18636 (Mix-batch 702-73-06-0001, 96.0 - 96.4% of purity) was administered in the diet to 60 male and 60 female Wistar rats per dose group, in doses of 0, 500, 2500 or 5000 ppm (main groups) for a scheduled treatment period of 2 years (carcinogenicity part of the study). Furthermore, BYH 18636 was administered in the diet to 10 male and 10 female rats per dose group, in doses of 0, 200, 500, 2500 or 5000 ppm (satellite groups) for a treatment period of 1 year (chronic part of the study). Treatment resulted in the following mean daily test substance intake for main groups (averaged over the study period of two years and given in ascending dosages): 22.8, 115.2 and 234.0 mg/kg body weight for males and 29.9, 152.9 and 313.4 mg/kg for females. During their treatment period of one year mean daily test substance intake of satellite group animals was for the males: 10.6, 27.2, 136.4 and 268.60 mg/kg body weight and for the females: 13.2, 35.8, 176.7 and 366.6 mg/kg. The animals were regularly observed and weighed and food intake was determined. Furthermore, ophthalmological investigations (before the study start: all animals; after one year and at the end of the treatment period: all main group animals of the control and high dose groups), a functional observational battery and motor activity assessment (once in week 50 on animals of the satellite groups) as well as clinical laboratory investigations of blood and urine samples (after 3 months, 6 months and near the end of the first year of treatment from animals of the satellite groups and after 3 months, 6 months, 12 months, 18 months and near the end of the second year of treatment from animals of the main groups) were performed. At necropsy, selected organs were weighed and tissues were subjected to gross and histopathological investigations.

Microscopy of urinary sediment showed the presence of crystals in nearly all animals of the 5000 ppm group as well as in two males and two females of the 2500 ppm group after 78 weeks of

treatment. However, at the end of the treatment period no such crystals were observed in the sediment. Furthermore, histopathological investigation confirmed no evidence of treatment-related effects in the kidneys/urinary tract.

The investigations gave no evidence for treatment-related adverse effects. Furthermore, under the conditions described the administration of BYH 18636 to male and female rats did not produce any evidence of an oncogenic effect of the test substance.

Therefore, the present study established a No-Observed-Effect Level (NOEL) of 5000 ppm (corresponding to 234.0 or 313.4 mg/kg body weight/day) in males or females, respectively. The UK reviewer did not concur with the study author's conclusion and identified a possible effect on serum triglycerides. In particular, serum triglycerides were lower in the high dose in females at 6 months (39%, $p < 0.05$) 12months (41%, $p < 0.05$ and at 18 months (in the mid dose group – 49% and high dose group -59%, $p < 0.01$). Males were also decreased at 12 months (-58%, $p < 0.05$) and at 24 months (-46%, $p < 0.05$). The UK reviewer acknowledges that an effect on serum triglycerides is a conservative call.

Classification:
By United Kingdom reviewer: Totally reliable. Refer to Appendix I and for the discussion of a possible effect on triglycerides and for setting the NOAEL at 2500 and the LOAEL at 5000 ppm.

By HED: Acceptable/Guideline. The study satisfies the requirement for a series 870.4300 combined chronic feeding/carcinogenicity study in rats. HED does not consider that the differences in triglycerides showing possible decreases are of consistency or magnitude to be considered a toxic response and the NOAEL for the study is > 5000 ppm.

b) Mouse study (MRID 47070135)

Executive summary:

Groups of 60 male and 60 female C57BL/6J mice were fed diet containing 0, 200, 1000 or 4000 ppm of BYH 18636 (mix-batch 702-73-06-0001) for at least 28 weeks. After 28 weeks, 10 males and 10 females from each group allocated to the 28-week phase of the study were necropsied at the scheduled interim sacrifice. The remaining 50 animals/sex/group, allocated to the carcinogenicity phase of the study, continued treatment until the scheduled final sacrifice of the study after at least 78 weeks of treatment. The mean intake of BYH 18636 over 18 months was calculated to be 0, 29.2, 147 and 599 mg/kg/day in males and 0, 36.8, 185 and 758 mg/kg/day in females, at 0, 200, 1000 and 4000 ppm, respectively. Mortality and clinical signs were checked daily. Additionally, detailed physical examinations including palpation for masses were performed weekly throughout treatment. Body weight and food consumption were measured weekly for the first 13 weeks of the study, then monthly thereafter. Haematology determinations were performed at approximately 6, 12 and 18 months from designated animals. Where possible, blood smears were prepared from moribund animals just before sacrifice. All animals were subjected to necropsy, with selected organs weighed at scheduled interim and final

sacrifice. Designated tissues were fixed and examined microscopically. In addition, urinary bladder stones sampled from five males at 4000 ppm were analyzed for the presence of test substance.

At 4000 ppm:

The mortality incidence over 18 months was higher (44%) in males than in the corresponding control group (22%), the effect being statistically significant. The higher mortality rate was largely due to the presence of stones within the urinary tract. Treatment-related clinical signs consisted of an increased incidence of generalized soiled fur in both sexes and an increased incidence of skin lesions, principally in the anogenital region, abnormal penis and wasted appearance in males. Abnormal penis was not associated with any intrinsic histopathological findings, but was associated with chronic ulcerative dermatitis and/or an abscess in the preputial gland in the majority of cases at the microscopic examination. Mean body weight was reduced by 3% on Study Day 176 in males; this slight reduction in mean body weight continued thereafter resulting in a 5% lower body weight at the end of the study on Study Day 540, when compared with the controls. Mean cumulative body weight gain in males was statistically significantly reduced throughout most of the study, resulting in an overall mean cumulative body weight gain reduction of 15% by Study Day 540, when compared with the controls. Mean body weight and body weight gain were unaffected by treatment in females. Mean food consumption was comparable to the controls in both sexes. Haematology parameters were unaffected by treatment.

At the 28-week interim sacrifice there was no treatment-related organ weight, macroscopic or microscopic findings.

At 1000 and 200 ppm:

No treatment-related changes were observed in either sex.

The No Observed Effect Level (NOEL) was 1000 ppm for both sexes (equivalent to 147 mg/kg/day in males and 185 mg/kg/day in females).

Classification:
By United Kingdom: Totally reliable.
By HED reviewer: Acceptable/Guideline. This study satisfies the requirement for a series 870.4200 carcinogenicity study in mice.

c. Dog Study

Executive summary: (MRID No.: 47040133 (2007). (As prepared by the testing laboratory and the Bayer Corporation).

Technical grade BYH 18636 was administered in the diet to Beagle dogs (4/sex/dose) at dose levels of 0, 1000, and 4000 ppm for at least 370 days, and 8000 ppm for 21 days which was then reduced to 7000 ppm for males and females on study day 21, due to the presence of urinary

calculi in males. Since urinary calculi persisted in males treated at 7000 ppm, a washout period of 4 days was conducted for this group between study days 52 and 55, before continuation of the treatment at 6000 ppm starting on study day 56 for the remainder of the 12-month treatment period. Cageside observations were conducted daily, detailed clinical observations were conducted weekly, food consumption was measured daily, and body weights were taken weekly. Ophthalmic examinations were performed once pre-exposure and just prior to necropsy. Clinical chemistry, haematology, and urinalysis measurements were taken once pre-exposure and during study weeks 14, 27, 40 and 53. A gross necropsy was performed, organ weights were taken, and tissues were examined microscopically.

At 6000 ppm in males/7000 ppm in females: In males, calculi were observed in the urinary bladder and were associated with micro pathology findings in the bladder of slight to moderate transitional cell hyperplasia, slight congestion, slight haemorrhage, slight inflammation, minimal calculus, and/or moderate ulceration.

At 4000 ppm and 1000 ppm: No treatment-related effects were observed.

The NOAEL for this study is 4000 ppm for males, equivalent to 117 mg/kg bw/day, and 7000 ppm for females, equivalent to 200 mg/kg bw/day.

Classification: UK reviewer "totally reliable". The United Kingdom reviewer concurred with the study summary assignment of the NOAEL (4000 ppm) and LOAEL 6000/7000/8000 ppm) but also indicated that there were body weight effects in the high dose group.

HED Reviewer: Acceptable/Guideline. The study satisfies the guideline requirement for a series 870.4100 chronic study in dogs.

5. Mode of Action

There were no specific mode of action studies submitted for the association between chronic exposure to TCM and development of non-neoplastic kidney or bladder lesions and eventual low incidence of neoplasms. The Registrant, however, presented a document entitled "An Evaluation of the Carcinogenicity of Thiencarbazone Methyl (BHY 18636) prepared by Samuel Cohen, MD, Ph.D. and dated September 12, 2007. A copy of this document is attached. Overall, thiencarbazone methyl was demonstrated to have the kidney/bladder and urothelial tissue as a target across species and urothelial toxicity is considered a well established mode of action for induction of hyperplasia and eventual neoplasia. TCM, like many sulfonamides, when ingested at high exposure levels, produce urinary tract solids, including calculi/stones, which lead to cytotoxicity, consequent inflammatory reaction and regenerative urothelial proliferation, and eventually progressing to tumors. The carcinogenic stimulus is not the chemical itself, but rather, tumors are associated with the toxicity and regenerative proliferation associated with the formation of urinary tract crystals and calculi. Thiencarbazone methyl is not considered to be mutagenic.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

Rat

• There were no significant treatment-related increases in tumors in male or female Wistar rats.

• Adequacy of Dosing: No overt toxicity was seen up to the highest dose tested of 5000 ppm (234 and 313.4 mg/kg/day in males and females, respectively), aside from a possible decrease in serum triglycerides, raising a question that the doses were inadequate for carcinogenicity assessment. However, based on the results of the subchronic study with TCM, the doses selected for the definitive carcinogenicity assessment are considered appropriate since there was significant toxicity at 7000 ppm in the rat subchronic study. This toxicity included treatment related mortality, clinical chemistry (increased alkaline phosphatase activity in males) and urinalysis (crystals in urine), as well as non-neoplastic changes including intrapelvic eosinophilic urolithiasis of the kidney and bladder and urothelial hyperplasia of the bladder. It is noted that an effect on serum triglycerides was not seen at the higher dose of 7000 ppm although the duration of exposure was for only 90 days. The CARC, therefore, concluded that the top dose of 5000 ppm in the cancer study was approaching an adequate dose based on the significant toxicity seen at 7000 ppm in the subchronic rat study. Furthermore, the CARC believed that testing at higher doses would not change the conclusions for assessing carcinogenicity.

Mouse

• *Males*

In males, the censored incidences of transitional cell tumors of the urinary bladder and urethra/prostate for the 0, 200, 1000, and 4000 ppm dose groups, respectively were as follows:

Bladder papillomas:	0/39 (0%), 0/34 (0%), 0/39 (0%), 1/28 (4%)
Urethra carcinomas:	0/38 (0%), 0/34 (0%), 0/37 (0%), 1/28 (4%)
Combined:	0/38 (0%), 0/34 (0%), 0/37 (0%), 2/28 (7%)

There were significant increasing trends for papillomas and carcinomas (both at $p<0.05$) and combined ($p<0.01$), as well as a significant difference in the pair wise comparison of the 4000 ppm dose group with the controls for the combined tumors, at $p<0.05$. These are considered to be rare tumors. Significant non-neoplastic pathology (hyperplasia and inflammation) occurred in males at the high dose. Therefore, the CARC considered the transitional cell tumors at the high dose to be treatment-related.

Females

• In females, the censored incidences of transitional cell tumors of the urinary bladder for the 0, 200, 1000, and 4000 ppm dose groups, respectively were as follows:

Papillomas: 0/45 (0%), 0/45 (0%), 0/46 (0%), 2/48 (4%)
Carcinomas: 0/45 (0%), 0/45 (0%), 0/46 (0%), 1/48 (2%)
Combined: 0/45 (0%), 0/45 (0%), 0/46 (0%), 3/48 (6%)

There was a significant increasing trend for combined tumors at $p<0.05$. These are considered to be rare tumors. Significant non-neoplastic pathology (hyperplasia and inflammation) occurred in females at the high dose. Therefore, the CARC considered the transitional cell tumors at the high dose to be treatment-related.

• Adequacy of Dosing: The CARC concluded that while the high dose may be an excessive dose, the study is considered adequate because the effects seen in both sexes at the high dose are typical of the primary target site (urothelial toxicity in the kidney and bladder).

2. Mutagenicity

• There is no mutagenicity concern for TCM or its major metabolites.

3. Structure Activity Relationship.

• TCM does not have a structural alert for genotoxicity. However, TCM is related to sulfonamides and sulfoamido compounds which have been shown to induce urinary bladder tumors at high doses similar to the response seen with TCM in the mouse study.

4. Mode of Action.

• The mode of action for the induction of the transitional cell tumors in the bladder in males (one incident), urethra/prostate tumor (one incident) and transitional cell papilloma (2 incidents) and carcinoma (one incident) in the bladder of females is considered to be related to the secondary effects of urothelial toxicity (irritation) and regenerative proliferation associated with the formation of urinary tract crystals/calculi in the urothelial structures at higher doses. This is a well established MOA for bladder tumors. TCM is not considered to be mutagenic.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005), the CARC classified Thiencarbazone-methyl as **"Not Likely to be Carcinogenic to Humans" at doses that do not cause urothelium cytotoxicity.** The formation of the low incidence of the transitional cell tumors of the bladder in both sexes and urethra/prostate in males seen at the high dose in mice is considered to be related to the secondary effects of the urothelial toxicity (irritation) and regenerative proliferation associated with the formation of urinary tract crystals/ calculi. This is a common mode of action for bladder carcinogenesis in rodents for non-genotoxic chemicals. No tumors were seen in rats. There is also no concern for mutagenicity.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantification is not required. The cRfD would be protective of both cancer and non cancer effects.

VIII BIBLIOGRAPHY

MRID No. CITATION

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47070127 Langrand-Lerche, C. BYH 18636 - 90-day toxicity study in the mouse by dietary administration, Bayer CropScience SA, Sophia Antipolis, France, Bayer CropScience AG, Report No.: SA 03086, Edition Number: M-000411-01-2. Date: 27.02.2004. GLP, unpublished

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Appendix 1

1. Carcinogenicity Evaluation Prepared by Dr. Samuel M. Cohen (September 12, 2007)

Bayer CropScience

An Evaluation of the Carcinogenicity of Thiencarbazone Methyl (BYH 18636)

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Thiencarbazone Methyl (TCM) is a sulfonamide – related chemical being developed as a pesticide. As part of the toxicity evaluation, long term carcinogenicity bioassays were performed in rats and mice. Shorter term assays were performed in rats, mice, and dogs. No tumorigenic effect was observed in rats in a two year bioassay, but there was a low incidence of urinary bladder tumors in mice in eighteen month study. I did not review the histopathologic slides, relying on the diagnoses of the study pathologists. Since these are straight forward types of lesions to evaluate, I feel comfortable in relying on their diagnosis. Also, the lesions that they describe are those typical of rodents with urinary tract crystals or calculi.

Bayer CropScience at my request has provided the following reports to me for this evaluation: A presentation entitled, "BYH18636-Thiencarbazone Methyl-Toxicological Update and Phys/Chem Data. July 2007" (23 pages); a document entitled, "Tier Two, Summary of Physical and Chemical Properties of the Active substance Thiencarbazone Methyl (BYH18636)" (24 pages); and a document entitled, "Tier Two, Summary of the Toxicological/Toxicokinetic Studies for Thiencarbazone – Methyl" (280 pages). These documents provided all of the information needed for my evaluation.

Data on Thiencarbazone Methyl

The carcinogenicity study in rats involved administration for two years at 0, 500, 2500, and 5000 ppm of the diet. In the mouse study, the doses evaluated were 200, 1000, and 4000 ppm in the diet,

administered for 18 months. In both species, males and females were tested. In the mouse, 1/50 males and 3/49 females had transitional cell tumors of the urinary bladder and 1/50 males had a transitional cell tumor of the prostatic urethra. In males, the bladder tumor was a transitional cell papilloma and the prostatic urethral tumor was a transitional cell carcinoma. In females, two of the urinary bladder tumors were papillomas and one was a transitional cell carcinoma. Furthermore, in the mouse there were high incidences of urothelial hyperplasia at the highest dose, with the incidences somewhat higher in males than in females. There were also high incidences of urinary tract stone formation in both the males and females, again with higher incidences in the males than in the females. The stones were associated with an inflammatory infiltrate and urothelial hyperplastic response. Calculi were also associated with obstruction leading to effects on the ureters and kidney pelves and consequent effects on the kidney parenchyma. Some of the calculi were analyzed, and the composition was 70-75% parent compound. All of these urinary tract effects occurred only at the high doses and were all associated with formation of calculi.

Although the incidences of bladder tumors were small in the mouse oncogenicity study, the high incidences of bladder hyperplasia justifies the conclusion that these were treatment related and did not occur by chance. It is also clear from these carcinogenicity studies that the carcinogenic effect is weak and occurs only at the highest dose. The carcinogenic stimulus is not the chemical itself, but rather, tumors are associated with the toxicity and regenerative proliferation associated with the formation of urinary tract calculi and crystals. These toxic manifestations of the compound were also observed in short-term studies in rats, mice, and in dogs. This is a common mode of action for bladder carcinogenesis in rodents for non-DNA reactive (non-genotoxic) chemicals (1-4).

TCM has a been extensively evaluated in genotoxicity assays in vitro and in vivo, and gives consistently negative results. These included mutagenesis in the Ames test, chromosome aberrations in V79 cells, the HPRT mutagenicity assay in V79 cells, and the in vivo micronucleus test in mice. Furthermore, various metabolites were also evaluated in genotoxicity assays and gave consistently negative results. These in vitro assays included negative results with and without metabolic activation systems added. In shorter term assays, including a period as short as three months and as long as one year, a high dose of the compound administered orally to rats, mice, and dogs produced similar toxicity. This toxicity included formation of urinary tract calculi and crystals, with consequent toxicity to the urothelium and occasionally obstruction. The urothelial toxicity was associated with an inflammatory reaction and regenerative hyperplasia. When calculi caused obstruction, the usual consequences of ureteral and kidney pelvis dilatation and ureteral and kidney pelvic inflammatory and proliferative lesions resulted. Toxicity only occurred in animals with calculi. Crystalluria was also evident in urine specimens collected in some of these studies in rats and dogs. Calculi were only present at the highest dose in any of these studies, but crystals were occasionally present in the urine of animals in the mid dose. The low dose was consistently negative for crystals, calculi, or other manifestations of urinary tract toxicity. It appeared that urinary tract toxicity only resulted if calculi formed, and that crystals were insufficient for producing the inflammatory and proliferative response of the urothelium.

In the long term mouse carcinogenicity assay, the urinary tract calculi were evaluated chemically for

their composition. The calculi were consistently composed predominantly of the parent compound, representing approximately 70-75% of mass. This is consistent with findings for a wide variety of sulfonamide chemicals that have been studied for more than four decades. TCM like other sulfonamides, is rapidly absorbed and excreted, with approximately half of the chemical excreted in the bile and feces and approximately half in the urine. It appears that most of the absorbed compound that is excreted in the urine is the parent chemical itself rather than metabolites. Metabolites which have been identified include the demethylated compounds and various hydrolysis products. Like most sulfonamides, TCM is only weakly soluble in aqueous solution, so it is quite consistent that the high concentration obtained in the urine after high dose oral administration would be more than sufficient to generate the crystals and calculi that were observed.

TCM produced a low incidence of bladder tumors in mice, but no urinary tract tumors in rats. In rats, crystalluria and calculi occurred in response to administration of TCM at high doses (generally greater than 2500 ppm). These were observed in short term studies of ninety days, and crystals were also seen in urine collections during the two year carcinogenicity assay at eighteen months. However, for reasons that are unclear from the study, crystalluria was not evident before the terminal sacrifice at twenty-four months. It may well have been that the consumption of the chemical on a weight basis had diminished to a low enough level that the amount being excreted in the urine was not sufficient for formation of the crystals. Regardless of the reasons for the disappearance of the crystals, it readily explains why there was no carcinogenic effect seen in the rat. It has been well demonstrated in studies with other chemicals that form urinary tract calculi that the proliferative response, even when extreme, such as to the extent of diffuse papillomatosis, is rapidly reversible, so that the bladders return to normal within one to three weeks after calculi are no longer present. Thus, if the rats did have hyperplastic changes of the urothelium through eighteen months of the study, these would have been rapidly reversible once the crystals and calculi no longer appeared. If the hyperplasia disappears, then tumors will not result.

In summary, TCM induced a low incidence of bladder tumors in mice, but not in rats. This occurred only at high doses and was associated with the formation of urinary tract calculi. Urinary tract calculi were a toxicologic response only at high doses in rats, mice, and dogs, and are consistent to what is known chemically, metabolically and toxicologically for sulfonamide and related compounds (5-7).

In assessing potential carcinogenic risk of TCM to humans, not only are the findings from studies with the chemical valuable, but the vast literature available for the effects of urinary tract calculi and specifically for sulfonamides provides extensive and valuable information to lead to the conclusion that not only does TCM not pose a carcinogenic risk to humans, but it does not represent a carcinogenic hazard for humans.

Examples of other compounds causing urinary tract calculi

Chemicals that produce urinary tract calculi in rodents can act as urinary bladder carcinogens, and occasionally produce tumors also in the ureters and kidney pelves (1-4, 8-10). The mode of action is

well characterized, involving administration of doses of chemicals that are high enough to generate a concentration of the material in the urine that can precipitate to form urinary tract crystals and/or calculi. These urinary solids produce a toxic effect on the urothelial mucosa (the urothelium), primarily in the bladder since that is where most of the calculi accumulate (11,12). Depending on the coarseness of the calculi, their size and number, variations in extent of damage and proliferation occur (13). This can also be influenced by dietary manipulations which can alter the chemical composition of the urine affecting the formation of the urinary solid, such as altering pH or concentration of the chemicals involved in the formation of the crystals (4,14).

Urinary solids can be formed from the parent compound or their metabolite, or solids can indirectly be formed from normal constituents of the urine because of dramatic alterations in the composition of the urine (10). An excellent example of the former that has been studied extensively is uracil (11,12). An indirect mechanism has been well described with the example of muraglitazar, a PPAR agonist, which produces hypocitraturia (15). Since citrate is the major chelating substance in the urine for calcium, a reduction in the level of citrate leads to the ready precipitation of calcium salts, such as calcium phosphate and calcium oxalate.

In the case of TCM, it appears that the urinary tract solids are formed primarily of the parent chemical itself rather than metabolites or from normal constituents of the urine. It is clear that it is not the chemical that is toxic, but rather, it is the urinary solid formed from the chemical.

Regardless of whether formed directly from the chemical or metabolite or indirectly from normal urinary constituents, urinary tract calculi represent a high dose phenomenon dependent on basic physical chemical properties of the solubility products of the chemical involved (1-4). This is the clearest example of a threshold phenomenon for carcinogenesis. In rodents, urinary solids can readily produce toxicity. This is especially true in male rats and mice compared to females, but both can be affected dependent on the toxicokinetics of the chemical in the specific gender and species.

Relevance for humans

The effects of urinary solids in humans are much less pronounced than in rodents (2-4). In contrast to rodents, urinary crystals do not appear to produce toxicity of the human urothelium (16-18). Crystals occur under a variety of circumstances in human urine, including normal human urine which contains magnesium ammonium phosphate (struvite) crystals and occasionally will also contain calcium phosphate or calcium oxalate crystals. Other crystals that form in human urine include uric acid and a variety of others that occur more rarely. Furthermore, a large number of drugs are also known to produce crystals in human urine, most notably, sulfonamides (5,6, 8-10). However, other classes of drugs also can produce crystalluria including carbonic anhydrase inhibitors and anti-HIV protease inhibitors (8-10). Crystalluria in humans does not represent toxicity, but may indicate a propensity for the formation of calculi (such as calcium oxalate or calcium phosphate) or may represent a manifestation of systemic toxicity, such as in the instance of uric acid in patients with gout (16).

Calculi are rarely present in the human urinary tract for prolonged periods of time (17). The human urinary tract anatomy differs from that of rodents so that urinary tract calculi usually produce obstruction and consequent severe pain, requiring immediate removal, either by lithotripsy or by surgery for those not fortunate enough to have the calculi dissolve with increased liquid intake (17). Obstruction usually occurs at the narrowing of the renal pelvic-ureteral junction, at the site where the ureter crosses the bony pelvic brim or at the bladder-urethral junction. In contrast, rats and mice can retain calculi in the bladder for long periods of time, primarily because the animal is horizontal and calculi can accumulate within the dome without producing obstruction. If obstruction does occur, it is most frequently partial and therefore not lethal to the animal.

In rodents, prolonged exposure to the calculus is required for tumors to occur (4,11,12). If the calculus is removed prior to the formation of malignant tumors, the proliferative lesions regress rapidly, even to the extent that papillomas can reverse. In humans, prolonged exposure to urinary tract calculi is uncommon.

Interpretation of this association is complicated by a variety of confounding factors. To begin with, many studies do not find a statistically significant relationship between calculi and the development of urothelial tumors (5,15,18). Secondly, many of the tumors that develop in patients with urinary tract calculi, like in other situations with prolonged urinary tract inflammation, is that the tumors are frequently squamous cell carcinomas rather than urothelial (transitional) cell carcinomas (19). In rodents, the tumors associated with calculi are preponderantly urothelial cell tumors. Lastly, and most importantly, prolonged urinary tract calculi in humans are uniformly associated with prolonged bacterial cystitis (20). Bacterial cystitis is a known risk factor for bladder cancer in humans, and the risk for developing tumors in patients with urinary tract calculi is somewhat less that in patients with bacterial cystitis without calculi, suggesting that the calculi do not add any additional risk to the development of urinary tract tumors compared to bacterial cystitis alone(20,21).

Additional evidence that urinary tract calculi do not represent a carcinogenic hazard in species other than rodents includes the observation that urinary tract calculi are extremely common in dogs, whereas urothelial tumors in dogs are uncommon (22).

Taking this information together, it strongly suggests that urinary tract calculi do not pose a carcinogenic hazard for humans, similar to dogs. Furthermore, for the case of TCM, there is a long experience with the toxicology and biology in humans is known for sulfonamide-related chemicals.

Sulfonamides and related chemicals are known to have poor solubility in aqueous solutions, such as urine (5-7). When ingested orally, they are rapidly absorbed and excreted primarily in the urine or in the urine and feces, as is the case for TCM. In the urinary tract, depending on solubility and various other factors affecting urinary chemical composition, urinary crystals are readily formed and in some species, also calculi (5-7). In rodents, as in the case for TCM, crystals and calculi readily form (5-7). For some sulfonamides, such as TCM, this also is true for the dog. As mentioned above, urinary tract calculi do not represent a carcinogenic hazard in dogs.

In humans, sulfonamides have been used clinically for more that fifty years, and their toxicologic effects are well known (23). This includes the usual formation of sulfonamide-containing crystals in the urine of patients on sulfonamides, without any adverse toxicologic consequence (5). Calculi can form from sulfonamides in humans but this is exceedingly rare and has not been associated with the formation of bladder tumors. In fact, many patients with long standing bacterial cystitis with or without urinary tract calculi are treated with long term sulfonamide antibiotic therapy (24). Based on this long clinical experience, sulfonamides are not considered carcinogens for humans, and are widely prescribed.

A similar non-DNA reactive consideration was given by the International Agency for Research on Cancer for another chemical, melamine, that produces urinary bladder tumors secondary to calculi, but in this instance in rats (25). Melamine, is classified by IARC in their monograph series on evaluation of carcinogenic risk of chemicals as a class three substance (the agent is not classifiable as to its carcinogenicity, based on mechanism). This was based on the fact that it is not only a high dose phenomenon, but exposure in humans is several orders of magnitude less than what is necessary for producing calculi and tumors in rats, and the likelihood that bladder tumors would result from calculi in humans is exceedingly low (as described above).

The lack of a relationship between urinary tract calculi and bladder cancer in humans is consistent with practical considerations of exposure to substances that can lead to the formation of calculi. For example, the most common calculi that occur in humans contain calcium, either as the oxalate or phosphate salt (26). In rodents, these produce urinary tract tumors. Calcium is an essential dietary ingredient, involved in many cellular, physiologic and structural functions (27). Calcium is present in many of the foods we consume, is used as a packaging ingredient for many food additives and drugs, and is an active ingredient in some drugs. There is no concern about its carcinogenic potential. In fact, there is accumulating evidence that it acts to prevent some types of cancer, such as colon carcinoma (28).

Summary and conclusion

In summary, TCM, like many sulfonamides, when ingested at high exposure levels, produces urinary tract solids, including calculi, which leads to cytotoxicity, consequent inflammatory reaction and regenerative urothelial proliferation, and in the mouse, rarely leads to bladder tumors. Based on the lack of genotoxicity, the well known biology and toxicology of urinary tract calculi, and the lack of carcinogenic effect of sulfonamides in humans, even when significantly high doses leading to the formation of sulfonamide crystals in the urinary tract are formed, TCM does not pose a carcinogenic hazard or risk to humans.

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